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14. ABSTRACT

Ablation of snf5, *in vivo*, results in an increase in the number and type of sensory neurons that express the capsaicin receptor, TRPV1. Using a culture system, we have determined that soluble factor is released by snf5-/- Schwann cells that acts on sensory neurons to induce the expression of TRPV1. This factor is greater than 10K molecular weight and does not affect neuron survival. The increase in the immunoreactivity of TRPV1 is correlated with an increase in the expression of functional capsaicin-sensitive ion fluxes.

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Introduction.

Schwannomatosis does not affect longevity but it has profound effects on the patient's quality of life due to the presence of intractable pain. The cause of this pain is not known. Our hypothesis is that mutations in the SNF5 gene in Schwann cells and ganglionic satellite cells leads to enhanced pain sensitivity in peripheral sensory neurons (see Campana et al, 2007). Mutations in the human SNF5 gene are linked to schwannomatosis (Hulsebos et al., 2007; Boyd et al.,2008; Hadfield et al., 2008; Sestini et al., 2008; Patil et al., 2008). Mice homozygous for snf5 deletion are embryonic lethal while heterozygotes develop rhabdoid tumors and other malignancies (Roberts et al., 2000; Klochendler et al., 2000). We are using a tomoxifen inducible Cre-mediated recombination system driven by a mouse proteolipid protein-1 (*Plp1*) promoter (Plp1-cre/ESR1;Jackson Labs) to target the KO to Schwann and satellite cells. When this mouse is crossed with a floxed snf5 mouse, gene activity is reduced by >80% in the peripheral nervous system. A description of our aims for this project and our preliminary data follows.

Project Proposed Aims and Results.

We proposed 2 specific aims: 1.**Test the hypothesis that the loss of snf5 increases pain sensitivity by increasing the expression of the capsaicin receptor (TRPV1) in polymodal nociceptors and by inducing capsaicin-sensitivity in sensory neurons of other modalities.**

We have utilized a tomoxifin-inducible plpCre / snf5-flox/flox mouse to produce an *in vivo* targeted deletion of SNF5. We are using the expression of the capsaicin receptor, TRPV1, to estimate the number of nociceptors in sensory ganglia of these animals.

In experiments where we acutely ablated snf5 in adult mice, we detect an increase in the number of TRPV1-expressing neurons in the trigeminal and dorsal root ganglia (**figure 1)**.

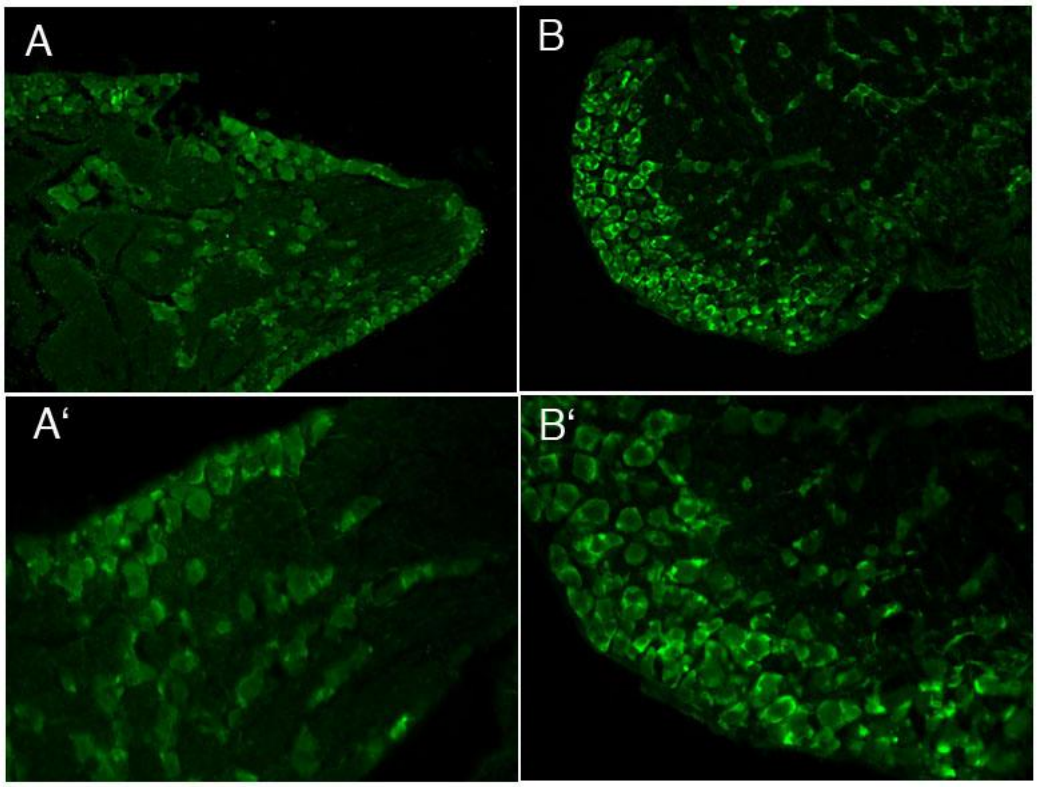

Figure 1. Trigeminal ganglion of oil injected (control; A,A') and tomoxifin injected (B, B') adult animals. Ganglia fixed 7 days after injections and lableled with anti-TRPV1. Panels are at 10X (A,B) and 20X (A', B').

To quantify the increase in TRPV1-IR neurons, we serially sectioned lumbar DRG (L1-5) and counted the neurons, ± TRPV1-IR, in every third section. The average percent positive neurons in 5 ganglia/animal were compared using a student's t-test. The results of 4 animals (2 tomoxifin-injected and 2 oil injected controls) were significantly different, 68 ± 9 vs 42 ± 6% respectively; $p< 0.01$

Strikingly, in preliminary cell counts, we observe an increase in large diameter (>24μm; 47% in tomoxifin- vs 8% oil injected controls) TRPV1-IR sensory neurons in lumbar DRGs (not shown) and trigeminal ganglia (**figure** 1). Since the small diameter (<20 μm) neurons are the typical nociceptors, this result suggests that sensory neurons of non-pain modalities are being recruited to express a nociceptor property. Consistent with this *in vivo* result, we detect an increase in large diameter, TRPV1-IR sensory neurons in cultures treated with conditioned medium (see figure 2 and Aim 2 below).

2. **Test the hypothesis that the loss of snf5 in Schwann and satellite cells results in an increase in their production and secretion of factors that increase the expression of TRPV1 in sensory neurons.**

In our second model we used an *in vitro* sensory neuron system to screen the biological activity of soluble factors released into the medium by snf5-knockout Schwann cells. Dorsal root ganglion (DRG) neurons of neonate to postnatal day 18 animals were dissociated and plated at a density of approximately 1000 neurons / cm^2. The base culture medium consists of L15, modified for 5% CO_2 atmosphere, as described in Mains and Patterson, (1973) with 5% adult rat serum, antibiotics and 100 ng/ml of 7s NGF.

The base medium was supplemented with medium conditioned by Schwann cells (SC-CM) ± snf5 (ablated using cre-lentivirual infection). The SC-CM was collected from confluent Schwann cell cultures, and concentrated using Amicon Ultra centrifugal filters with a 10000 molecular weight cutoff (Millipore). The SC-CM±snf5 was tested at 1-5 X concentration for 48-96 hours.

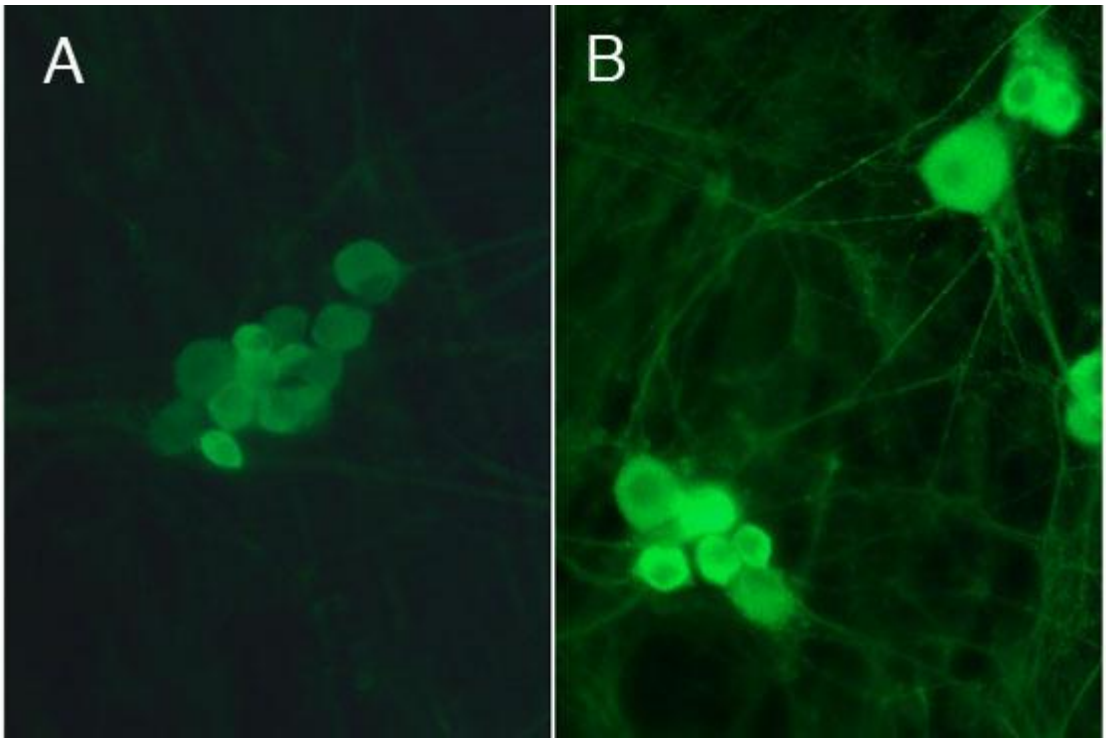

Figure 2. DRG culture treated with Control SC-CM (A) and -/- snf5 SC-CM (B) for 48 hours and labeled with anti-TRPV1. Note the presence of large diameter TRPV1-IR neurons in (B)

In these experiments, dissociated neurons were plated onto laminin-coated, 1 cm diameter glass coverslips. After CM treatment, the cultures were fixed and labeled with anti-TRPV1 antibodies and the appropriate secondary antibodies. For cell counts, each coverslip was divided into 4 sections and 3 randomly selected 20X fields were counted.

After 48 hours in CM we observe an increase in the percentage of TRPV1-IR neurons in cultures treated with -snf5 SC-CM (73 ± 7%) vs control SC-CM treatment (52 ± 2%). In addition, we observe both large and small diameter TRPV1-IR neurons in the snf5 -/-, SC-CM treatment but predominately small diameter neurons in the control SC-control CM, as we observed in the sensory ganglia *in situ* (not quantified at this time, figure 1)

We used a histochemical assay to determine the capsaicin sensitivity of sensory neurons co-cultured with ± *SNF5* SC-CM. In this assay, DRG cultures were incubated in a saline solution containing 0 calcium and 5 mM cobalt ± capsaicin (50 μM). After washing, intracellular cobalt was precipitated with ammonium sulfide. Following fixation, the cobalt precipitate was enhanced with silver using the Timm's intensification protocol (Matsumoto, 1994). Using this method, we detect a significant increase ($p < .01$) in capsaicin-sensitive neurons in –snf5 SC-CM (92 ± 1%) vs 54 ± 3% in control SC-CM treated cultures (figure 3)

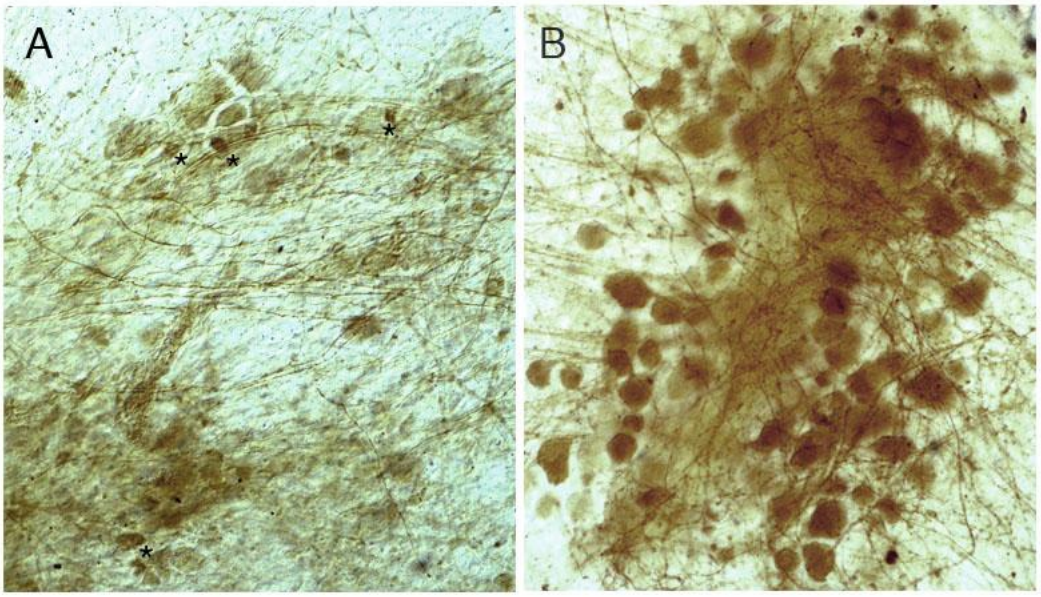

Figure 3. DRG culture, control SC-CM (A) and -snf5 SC-CM (B) treated with 50 µM capsaicin in the presence of 5 mM cobalt and subsequently enhanced with Timm's silver intensification protocol. Note, small diameter neurons labeled in (* in A) while many large and small diameter neurons labeled in (B).

We are currently preparing a microarray experiment to identify potential candidate molecules for the CM effect.

Key Research Accomplishments.

Target-deletion of SNF5 leads to increase TRPV1 expression in sensory neurons
SNF5 -/- Schwann cells secrete a factor that stimulates the expression of TRPV1
The conditioned medium factor increases the capsaicin sensitivity of multiple classes of sensory neuron.

Reportable Outcomes.

Manuscript in preparation

Conclusion.

The preliminary identification of a conditioned medium factor (CM) that is >10K mw is interesting and potentially very important towards advancing our understanding of this disease. We are preparing to run a microarray to identify potential candidate molecules mediating this activity. Our finding that the CM factor induces TRPV1 expression in large diameter sensory neurons is significant, since it suggests that one outcome of the mutation may be to convert non-pain sensory neurons to this phenoytype.

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